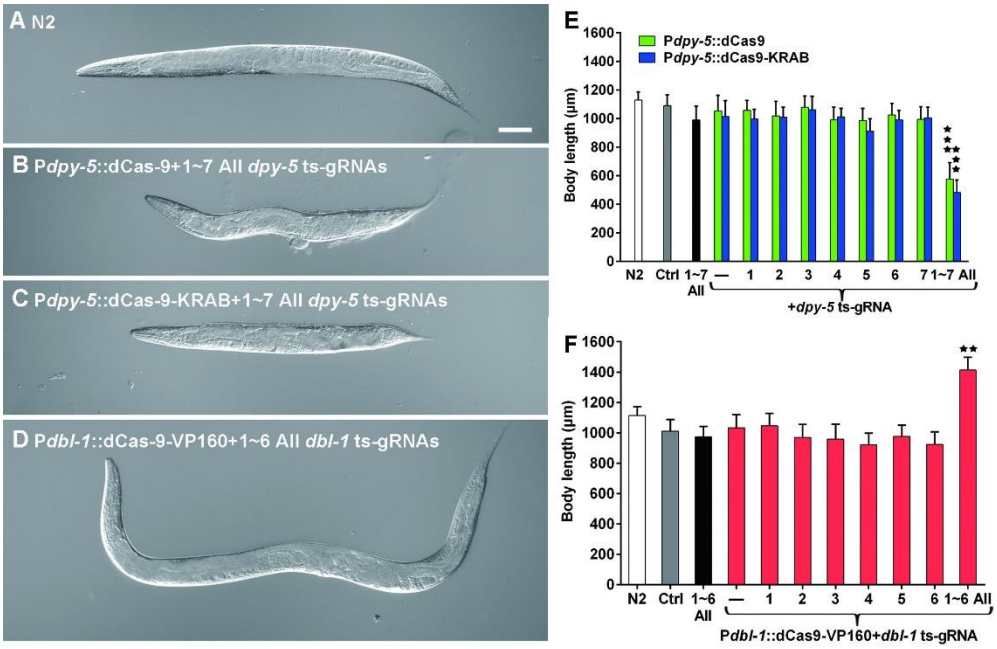

Figure S1a. The modular dCas9 fusion system works efficiently to suppress and activate endogenous gene expression in *C. elegans*

A, a normal wild-type hermaphrodite N2 is shown.

B, a *dpy-5*-supressed F1 worm (Dpy) is shown. It is from F1 progeny of transgenic founder (P0) worms injected with P*dpy-5*::dCas9 DNA and seven ts-gRNA plasmids (1-7 All).

C, a *dpy-5*-supressed F1 worm (Dpy) is shown. It is from F1 progeny of transgenic founder (P0) worms injected with P*dpy-5*::dCas9-KRAB DNA and seven ts-gRNA plasmids (1-7 All).

D, a *dbl-1*-activated F1 worm (Lon) is shown. It is from F1 progeny of transgenic founder (P0) worms injected with P*dbl-1*::dCas9-VP160 DNA and six ts-gRNA plasmids (1-6 All).

E, the measurement of average body length of the *dpy-5*-supressed worms is summarized. It reveals the requirement of multiple ts-gRNA sites in suppressing *dpy-5* expression. N2: wild-type (the white bar), Ctrl: P*col-10*::mCherry (the grey bar) and 1-7 All: all seven ts-gRNA plasmids in *pRF-4*[rol-6(su1006)] worms (the black bar). The ts-gRNA plasmids containing none, single target site (one of seven target sequences) or all seven sites (1-7 All) were co-expressed with P*dpy-5*::dCas9 or P*dpy-5*::dCas9-KRAB in N2 worms.

F, the measurement of average body length of the *dbl-1*-supressed worms is summarized. It reveals the requirement of multiple ts-gRNA sites in activating *dbl-1* expression. N2: wild-type (the white bar), Ctrl: P*col-10*::mCherry (the grey bar) and 1-6 All: all six ts-gRNA plasmids in *pRF-4*[rol-6(su1006)] worms (the black bar). The ts-gRNA plasmids containing none, single target site (one of six target sequences) or all six sites (1-6 All) were co-expressed with P*dbl-1*::dCas9-VP160 in N2 worms.

Each bar in E and F is displayed as mean ± SEM of three independent experiments and normalized to wild-type N2. The scale bar is 100μm.

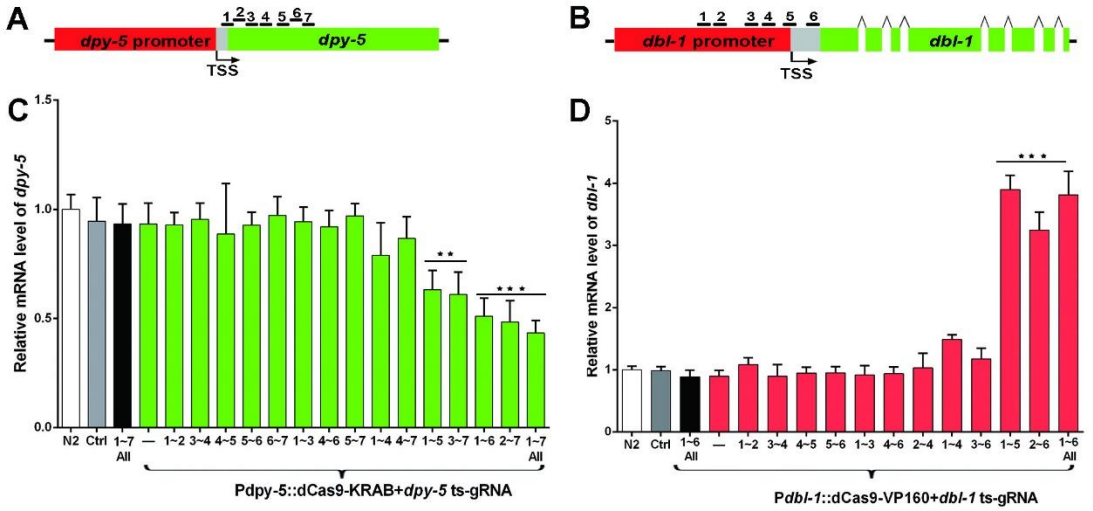

Figure S1b. Multiple sgRNAs are necessary for efficient knock-down and over-expression in C. *elegans*.

A, shown is *dpy-5* locus, where TSS, (arrow), ORF (green bar), and seven (1-7) *dpy-5*ts-gRNA targeting sites (short black lines) are indicated. All lines target the non-template DNA strand. The red bar represents *dpy-5* promoter. P*dpy-5*::dCas9 or P*dpy-5*::dCas9-KRAB was co-expressed with individual ts-gRNA plasmid (one of seven ts-gRNAs) or all seven ts-gRNA plasmids (1~7 All) in N2 worms.

B, shown is *dbl-1* locus, where TSS (an arrow), ORF (green bar), and six (1-6) *dbl-1*ts-gRNA targeting sites (short black lines) are indicated. Lines 1, 2, 3, 4 and 6 target template DNA strand of *dbl-1*, while line 5 targets template DNA strand. Red bar represents *dbl-1* promoter and spaces in green bar represent seven *dbl-1* introns. P*dbl-1*::dCas9-VP160 was co-expressed with individual *dbl-1*ts-gRNA (one of six ts-gRNAs) or all six ts-gRNA plasmids (1~6 All) in N2 worms.

C, qRT-PCR results reveal requirement of multiple sgRNA sites in suppressing *dpy-5* expression. N2: wild-type (the white bar), Ctrl: P*col-10*::mCherry (the grey bar), the combination of different ts-gRNAs and 1-7 All: all seven sgRNA plasmids in *pRF-4*[rol-6(su1006)] worms (the black bar).

D, qRT-PCR results also reveal requirement of multiple sgRNA sites in activating *dbl-1* expression. N2: wild-type (the white bar), Ctrl: P*col-10*::mCherry (the grey bar) and 1-6 All: all six sgRNA plasmids in *pRF-4*[rol-6(su1006)] worms (the black bar). Total RNA was isolated from injected or un-injected embryos at 11hpf. Each bar is displayed as mean ± SEM of three independent experiments and normalized to the wild-type values (C-F)Student's t test, ** indicates $p < 0.01$; *** indicates $p < 0.001$.

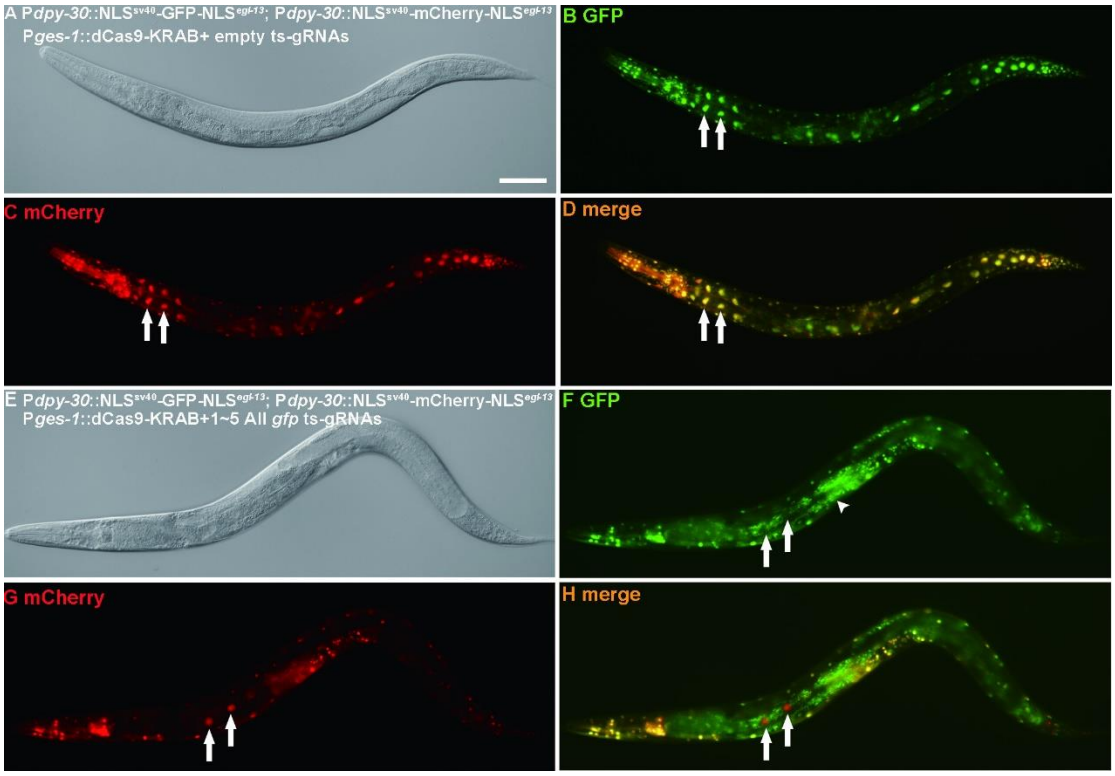

Figure S1c. Tissue-specific gene suppression by dCas9-KRAB and ts-gRNAs.
A-D, Live images of transgenic worm, ubiquitously expressing both GFP and mCherry, injected by P*ges-1*::dCas9-KRAB and ts-gRNAs (empty vector). DIC (A), green channel (B), FITC channel (C) and merged of B and C (D). Arrows indicates the intestine unclei. Scale bar is 100μm.
E-H, The double transgenic worms were injected by P*ges-1*::dCas9-KRAB and 1~5 All *gfp*ts-gRNAs. Arrows indicate the intestine nuclei, and the arrow head indicates intestinal auto-fluorescence.

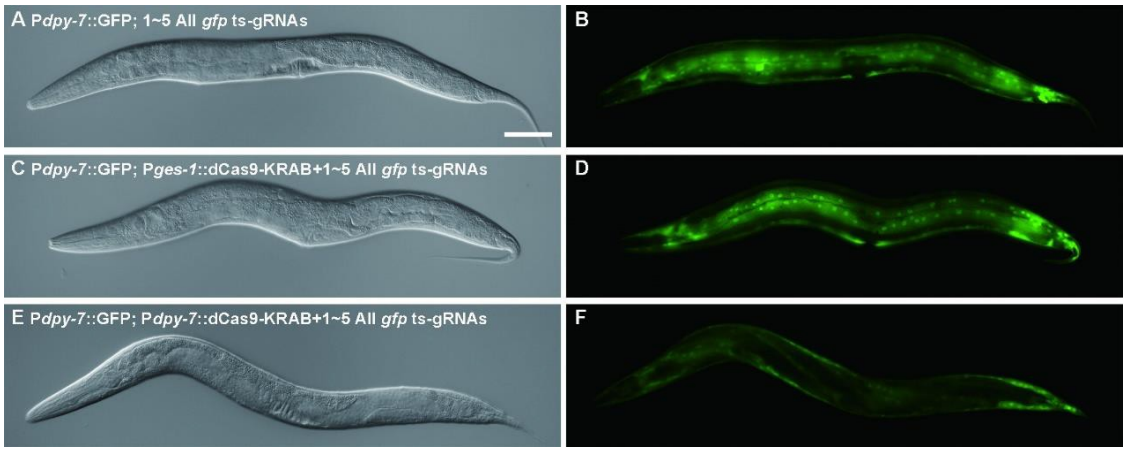

Figure S1d. dcas9 repress gene expression in a cell-autonomous manner.

A-**B**, transgenic worm, P*dpy-7*:: GFP; P*U6*::1~5 *gfp*ts-gRNAs, shows GFP expression in hypodermal cells normally. Scale bar is 100μm.
C-**D**, transgenic worm, P*dpy-7*:: GFP; P*ges-1*::dCas9-KRAB; P*U6*::1~5 *gfp*ts-gRNAs, shows GFP expression is unchanged when dCas9-KRAB is present in the intestine.
E-**F**, transgenic animal, P*dpy-7*:: GFP; P*dpy-7*::dCas9-KRAB; P*U6*::1~5 *gfp*ts-gRNAs, shows decreased GFP expression in hypodermal cells when dCas9-KRAB is driven by P*dpy-7* promoter.

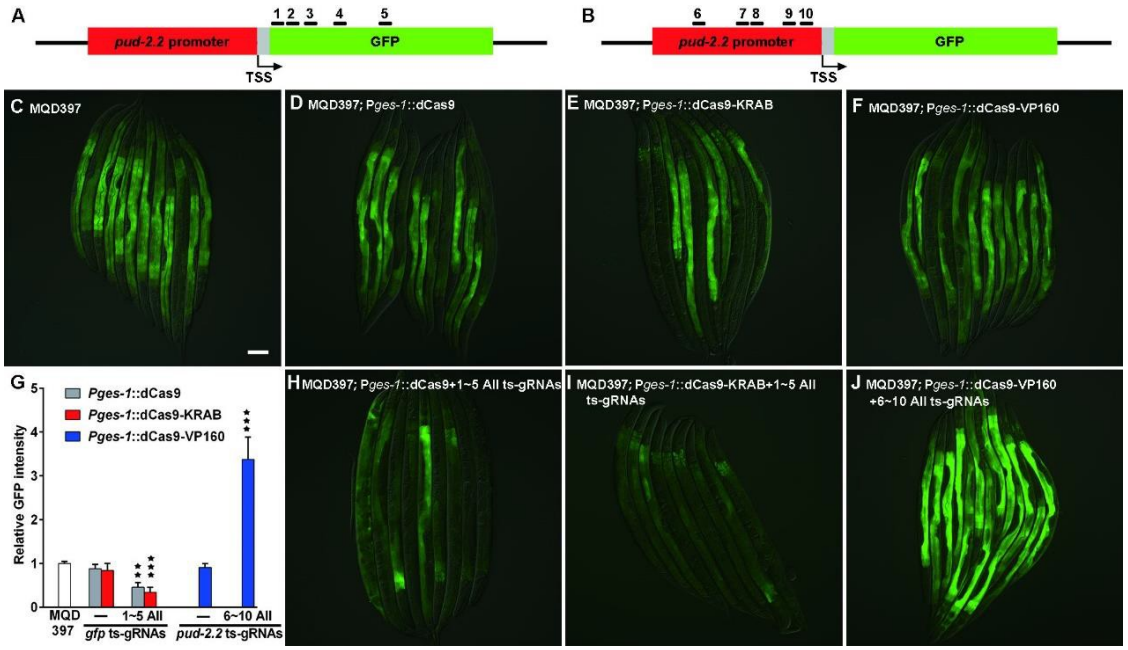

Figure S1e. Imaging the changes of GFP expression in F1 progeny from the injected P0.

A-B, shown in A is the transgene cassette in which five (1-5) sites (short black lines) target 5' UTR (1, 2) and ORF (3, 4, 5) of *gfp*, respective, while five sites target non-template strand (6, 8, 10) and template strand (7, 9) of *pud-2.2* promoter, respectively (B). TSS (arrow), *gfp* ORF (green bar), and *pud-2.2* promoter (red bar) are shown.

C-J, compared to control (MQD397) worms (C), P*ges-1*::dCas9 (D), P*ges-1*::dCas9-KRAB (E) or P*ges-1*::dCas9-VP160 (F) alone does not affect endogenous *gfp* significantly, and this conclusion is also judged by GFP intensity measurements (G). When these suppression or activation constructs are co-expressed with (1-5 All) or (6-10 All) ts-gRNAs, down-regulated (H, I) or elevated (J) *gfp* expression is evident. In all qRT-PCR experiments, relative values equal to means±SEM of three independent experiments normalized to wild-type/control animals. *p* values were calculated using unpaired Student's t-test. * indicates $p<0.05$; ** $p<0.01$; and *** $p<0.001$.The scale bar is 200μm.

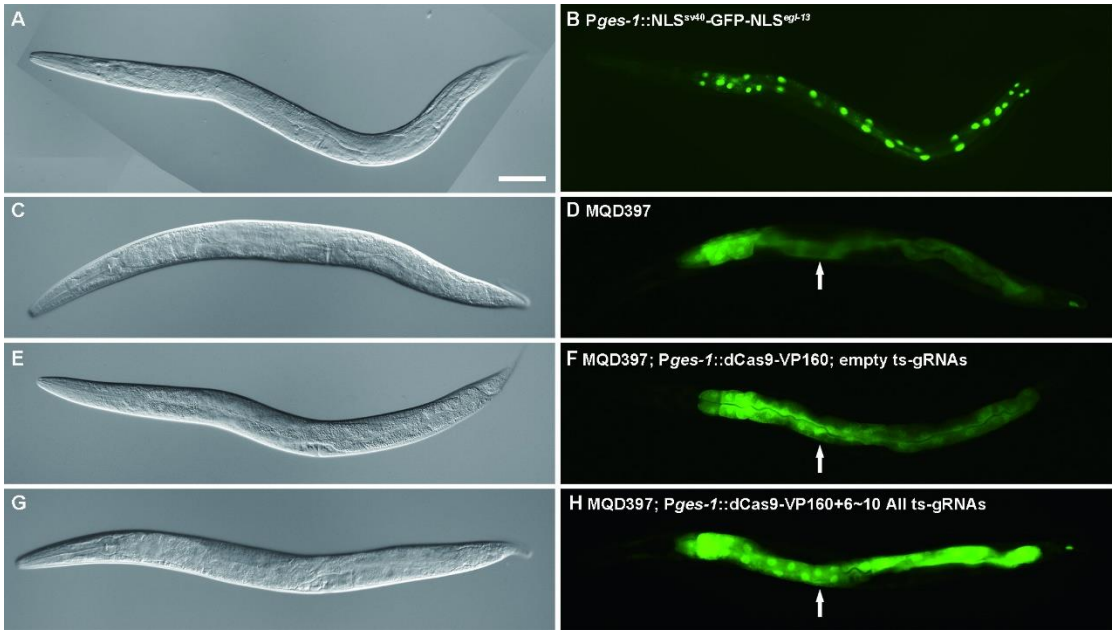

Figure S1f. **Intestine-specific *ges-1* promoter enhances GFP expression only in the intestine.**

A-**B**, *ges-1* promoter drives intestine-specific GFP expression.

C-**H**, in control worms (MQD 397), GFP is observed mainly in the intestine (C-D). While in the presence of P*ges-1*::dCas9-VP160 and all five *pud-2.2*ts-gRNAs(6~10) GFP is enhanced mainly in the intestine (G-H), otherwise, the enhancement is minimal without any *pud-2.2*ts-gRNAs (E-F).

The scale bar is 100μm. Arrows point to areas where intestinal cell nuclei are visible.

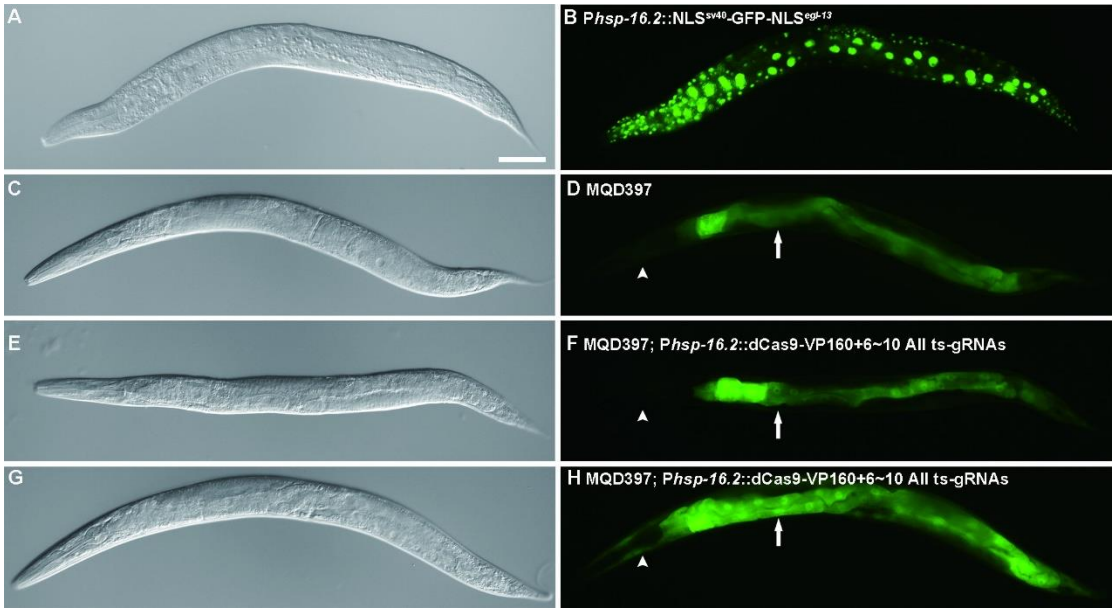

Figure S1g. ***hsp-16.2*** **promoter drives ubiquitous GFP expression.**

A-B, When transgenic worm (PLD2017, mfdEx2017[P*hsp-16.2*::NLS$^{2\times SV40}$-GFP-NLS^{egl-13}-unc-54 3UTR; Pcol-10::mCherry) is heat shocked at 35℃, GFP is detected in every cell. The scale bar is 100μm.

C-D, in control worm (MQD 397), GFP is observed mainly in the intestine (arrow) and weakly in the epidermis.

E-F, without heat shock, in the presence of P*hsp-16.2*::dCas9-VP160 and all five *pud-2.2*ts-gRNAs(6~10), transgenic animals show no change in GFP expression compared with control worm (MQD 397)

G-H, upon heat shock, GFP expression is enhanced significantly in the intestine (arrow) and epidermis (arrowhead).

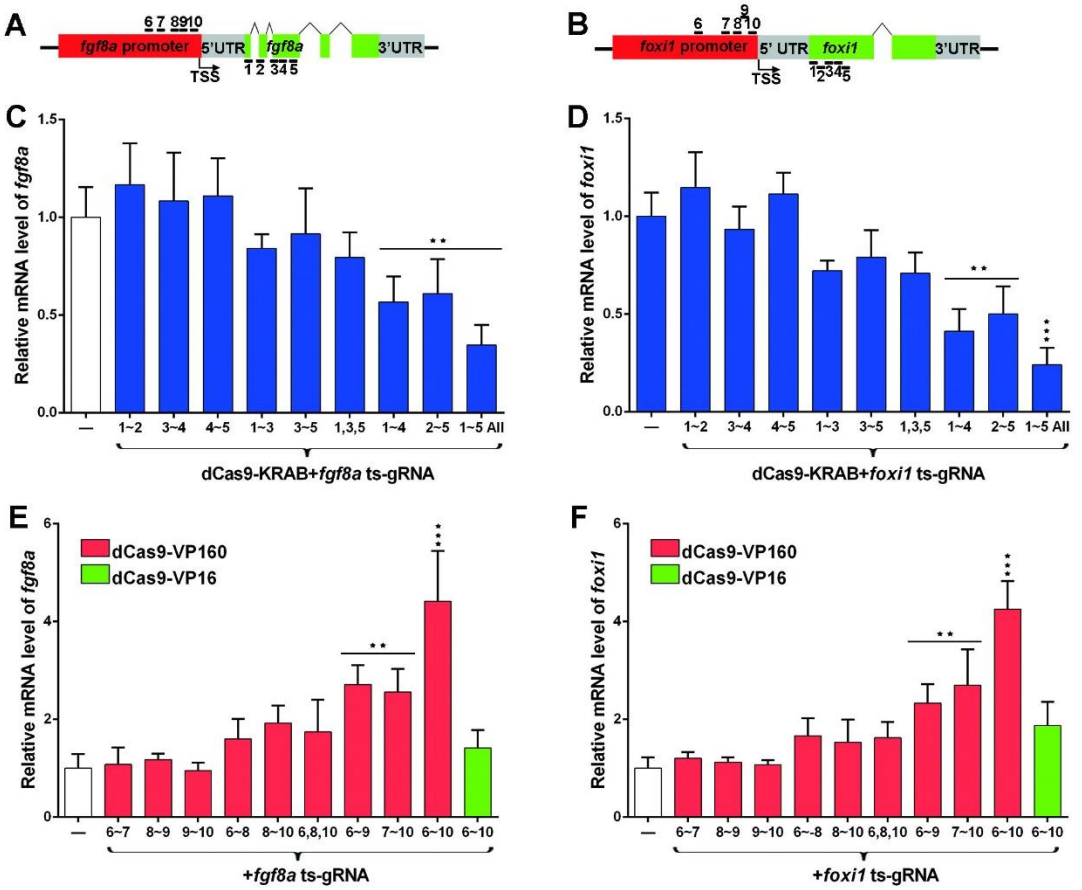

Figure S1h. Multiple sgRNAs are necessary for efficient knock-down and over-expression in zebrafish.

A-B, shown is zebrafish *fgf8a* (A) or *foxi1* (B) locus, where TSS (arrow), ORF (green bar interrupted by intron(s)), and five (1-5) ts-gRNA targeting sites (short black lines below) are indicated. The red bar represents the *fgf8a* or *foxi1* promoter and short black lines above are five (6-10) ts-gRNA targeting sites.

C-D, qRT-PCR experiments reveal requirement of multiple ts-gRNAs in suppressing *fgf8a* (C) or *foxi1* (D) expression: injecting the empty ts-gRNA scaffold, in the presence of dCas9-KRAB mRNA (the white bar), the combination of two, three or four ts-gRNAs. 1~5 All: injecting all five ts-gRNAs and dCas9-KRAB mRNA (the blue bar).

E-F, qRT-PCR experiments also reveal requirement of multiple ts-gRNAs in activating *fgf8a* (E) or *foxi1* (F) expression: injecting the empty ts-gRNA scaffold, in the presence of dCas9-VP160 mRNA (the white bar), the combination of two, three or four ts-gRNAs. 6~10 All: injecting all five ts-gRNAs and dCas9-VP160 mRNA (the red bar) or dCas9-VP16 mRNA (the green bar).

Total RNA was isolated from injected embryos at 11hpf. Each bar is displayed as mean ± SEM of three independent experiments and normalized to the wild-type values (C-F). Student's t test, ** indicates $p < 0.01$; *** indicates $p < 0.001$.

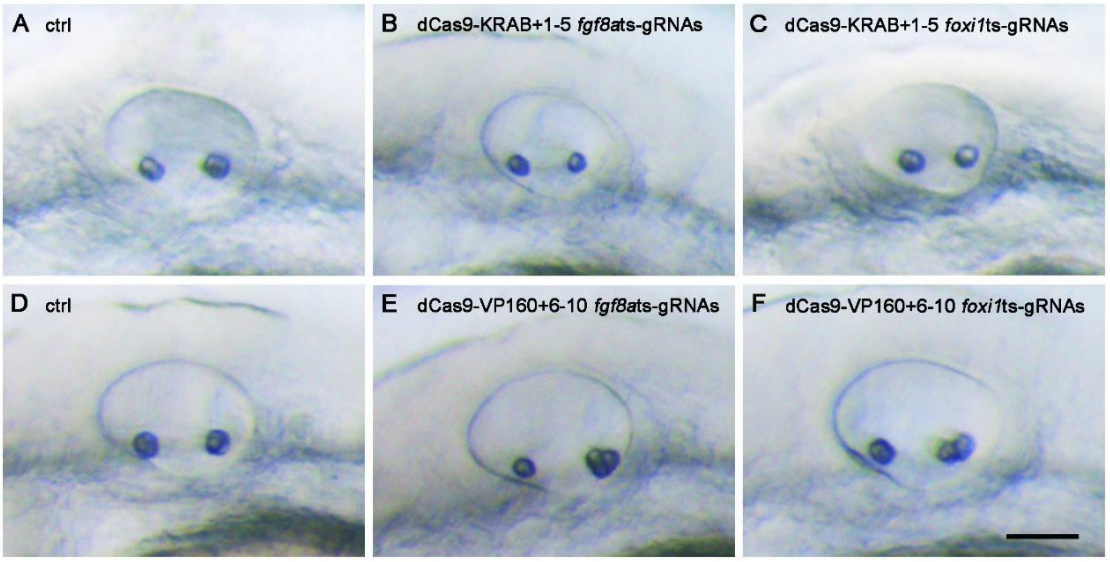

Figure S1i. **Otic development is affected by dCas9 fusion system-mediated regulation of *fgf8a* or *foxi1*.**

A-F, Lateral views of live embryo ears at 32hpf. Injected embryo with dCas9-KRAB mRNA and empty gRNA scaffold (A), and embryos injected with dCas9-KRAB mRNA and all 1~5 *fgf8a*ts-gRNAs or 1~5 *foxi1*ts-gRNAs (B, C). Injected embryo with dCas9-VP160 mRNA and empty gRNA scaffold (D), and embryo injected with dCas9-VP160 mRNA and all 6~10 *fgf8a*ts-gRNAs or 6~10 *foxi1*ts-gRNAs (E, F). The scale bar is 50μm.

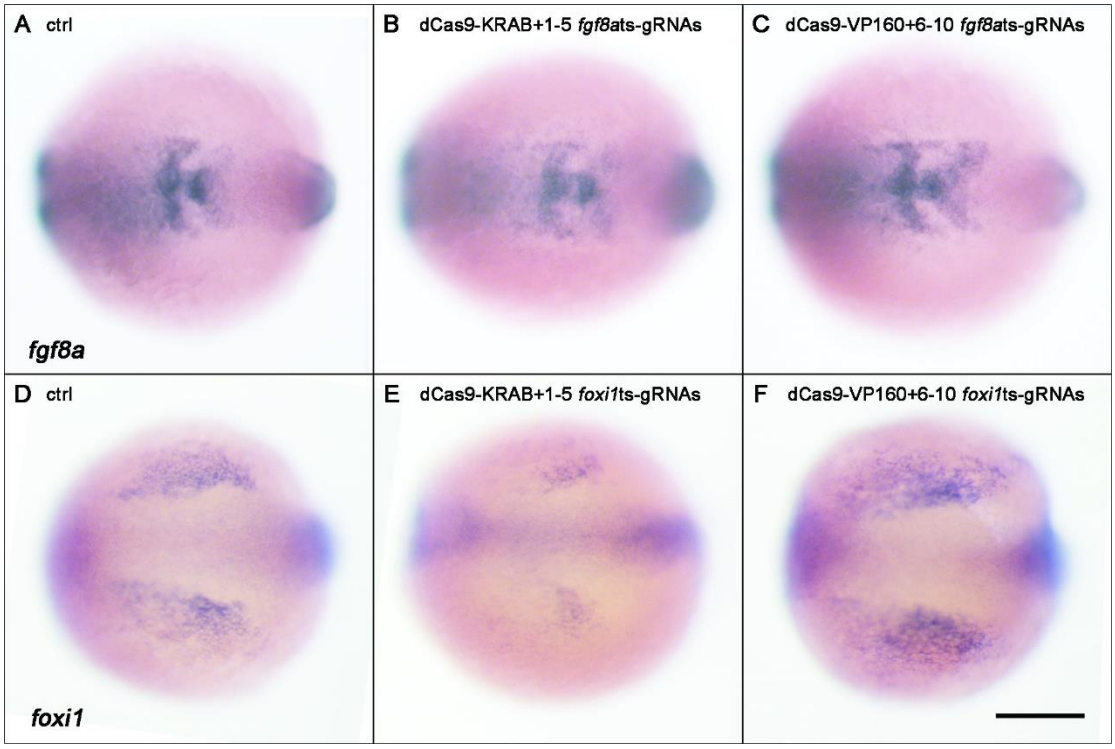

Figure S1j. Suppression and activation of endogenous genes using dCas9 fusion system

A-B, the dCas9 fusion systems work efficiently to regulate *fgf8a* during zebrafish embryogenesis. Compared to wild type (A, n=39), 71% embryos show decreased *fgf8a* expression (B, n=34) with dCas9-KRAB mRNA and 1~5 *fgf8a*ts-gRNAs, while 59% embryos show increased *fgf8a* expression by injecting dCas9-VP160 mRNA and 6~10 *fgf8a*ts-gRNAs (C, n=32).

D-F, the dCas9 fusion systems regulate *foxi1*. Compared to wild type (A, n=44), 68% embryos show decreased *foxi1* expression (B, n=40), while 54% embryos show increased *foxi1* expression (C, n=59).

In situ hybridization probe is anti-sense *fgf8a* or *foxi1* RNA. Dorsal views with anterior to right. 11.67hpf. The scale bar is 200μm.

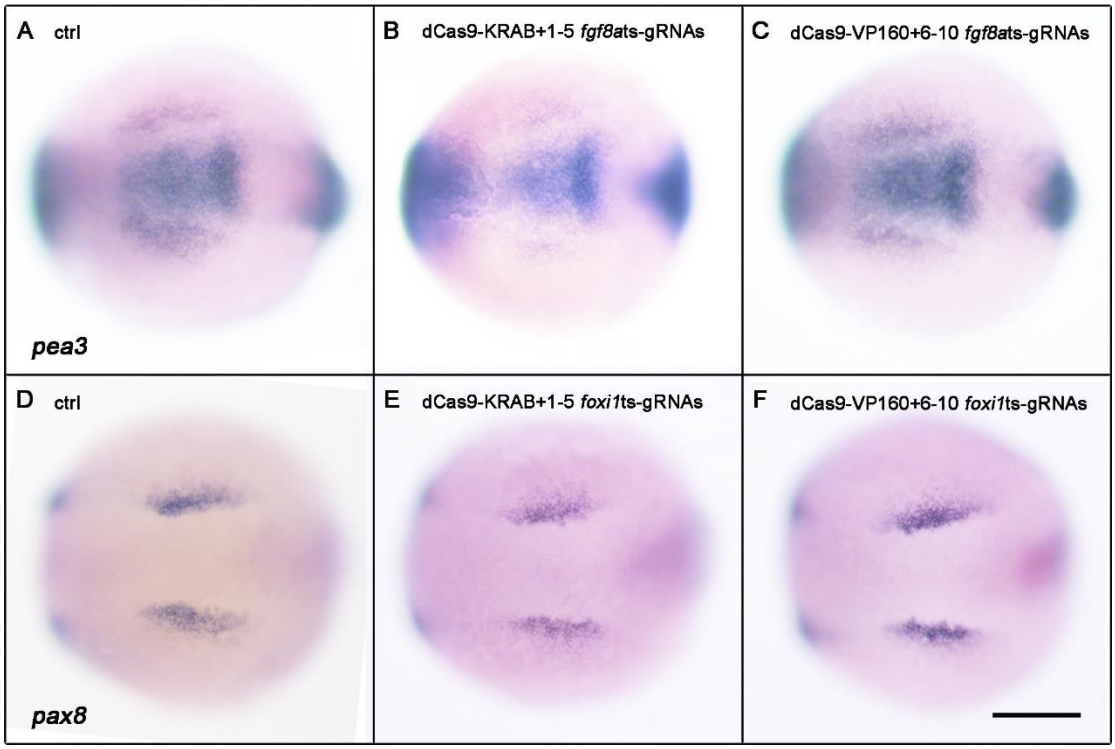

Figure S1k. Downstream genes of dCas9 fusion system-targeted endogenous genes are also affected.

A-C, *fgf8a* downstream gene *pea3* is also regulated accordingly. Compared to control (A, n=35), 75% embryos show decreased *pea3* expression (B, n=20) by injecting dCas9-KRAB mRNA and all 1~5 *fgf8a*ts-gRNAs, while 53% embryos show increased *pea3* expression by injecting dCas9-VP160 mRNA and all 6~10 *fgf8a*ts-gRNAs (C, n=38).

D-F, *foxi1* downstream gene *pax8* is regulated accordingly. Compared to control (D, n=27), 63% embryos show decreased *pax8* expression (E, n=33) by injecting dCas9-KRAB mRNA and all 1~5 *foxi1* ts-gRNAs, while 56% embryos show increased *pax8* expression by injecting dCas9-VP160 mRNA and all 6~10 *foxi1* ts-gRNAs (F, n=34). Dorsal views with anterior to right. 11.67hpf. The scale bar is 200μm.

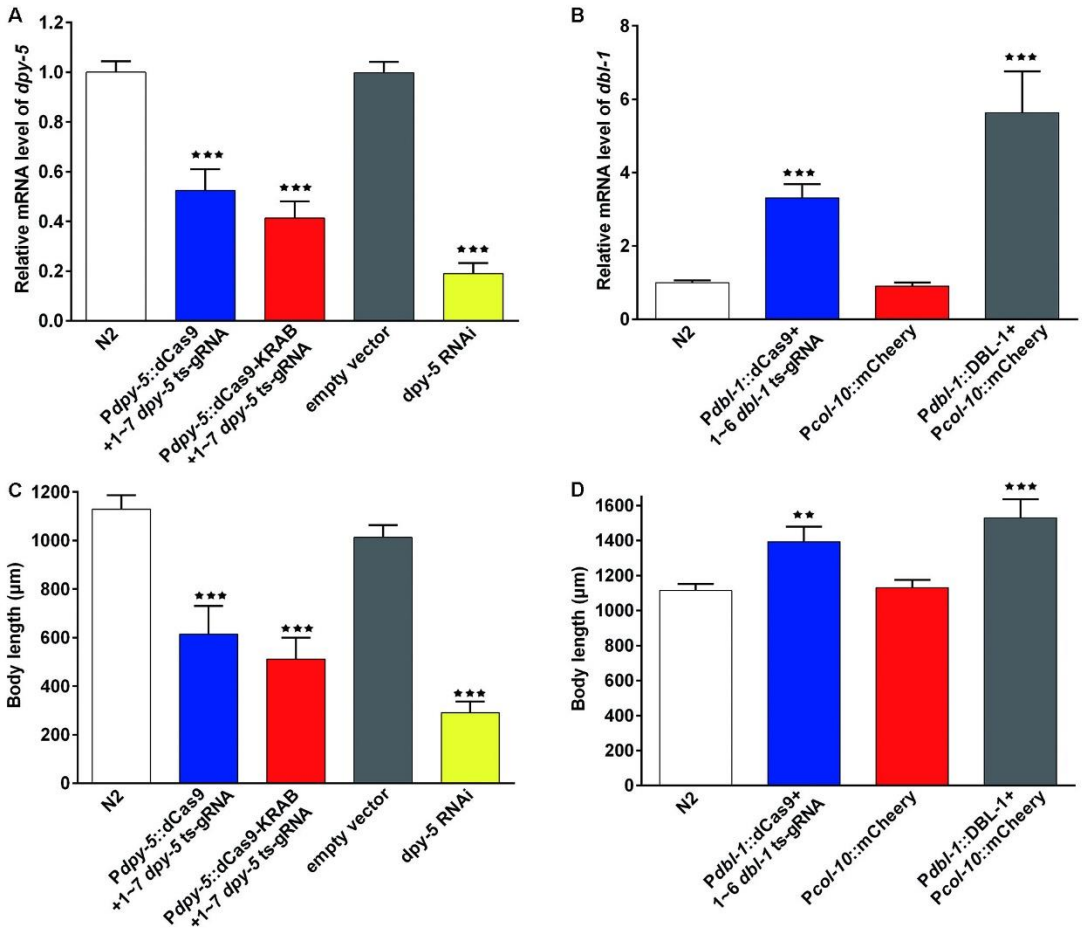

Figure S1l. Comparison of (suppressing and activating) dCas9 fusion system and other knocking down and mis-expression techniques in *C. elegans*

A and **C**, the reduction of endogenous *dpy-5* expression (A) and body length (C) in worms co-expressing dCas9 or dCas9-KRAB and seven ts-gRNAs (blue and red bars) is less severe than that of *dpy-5* RNAi (yellow bars). White bars: N2 wild-type worms and gray bars: the RNAi (empty) vector.

B and **D**, an increase of endogenous *dbl-1* expression (B) in worms co-expressing dCas9-VP160 and six ts-gRNAs (blue bar) is detected by qRT-PCR, compared to N2 worms. In P*col-10*::mCherry transgenic worms, the expression of *dbl-1* is normal (red bar). The *dbl-1* expression is increased significantly by P*dbl-1*::DBL-1 (grey bar). As to the body length measurements (D), the difference between dCas9-VP160 and six ts-gRNAs worms (blue bar) and P*col-10*::mCherry transgenic worms (grey bar) is less pronounced.

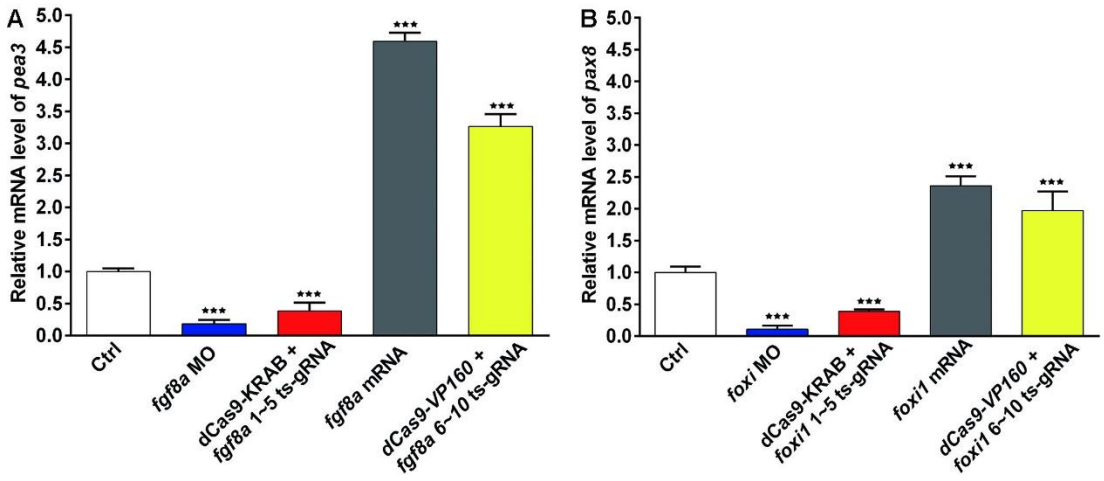

Figure S1m. Comparison of (suppressing and activating) dCas9 fusion system and other knocking down and mis-expression techniques in zebrafish embryos

A, the reduction of endogenous *pea3* expression in dCas9-KRAB and five ts-gRNAs (recognizing five sites of *fgf8a* coding region) (red bar) is comparable to that of *fgf8a*MO injection (blue bar). Ctrl: the un-injected wild-type embryos (white bar). The increase of endogenous *pea3* expression in dCas9-VP160 and five ts-gRNAs worms (yellow bar) is comparable to that of *fgf8a* mRNA injection (dark grey bar).

B, the reduction of endogenous *pax8* expression in dCas9-KRAB and five ts-gRNAs (recognizing five sites of *foxi1* coding region) (red bar) is comparable to that of *foxi1*MO injection (blue bar). Ctrl: the un-injected wild-type embryos (white bar). The increase of endogenous *pax8* expression in dCas9-VP160 and five ts-gRNAs (yellow bar) is comparable to that of *foxi1* mRNA injection (dark grey bar).

Each bar is displayed as mean ± SEM of three independent experiments and normalized to the wild-type values.